PB83-231241

Selenium Concentrations and Glulathione Peroxidase Activities in a Population Exposed to Selenium via Drinking Water

California Univ., Los Angeles

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

1980

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/J-80-410	2.
3. RECI	PB83-231241
4. TITLE AND SUBTITLE: Selenium Concentrations and Glulathione Peroxidase Activities in a Population Exposed to Selenium via Drinking Water (Journal Version)	5. REPORT DATE: 1980
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S): Valentine, Jane L., Kang, Han K., Dang, Phoung-Mai, Schluchter, Mark	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: School of Public Health, University of California, Los Angeles, California 90024	10. PROGRAM ELEMENT NO. 3BMCIA
	11. CONTRACT/GRANT NO. R-803798-01
12. SPONSORING AGENCY NAME AND ADDRESS: Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES: Valentine, Jane L., Kang, Han K., Dang, Phoung-Mai, Schluchter, Mark, Selenium Peroxidase Activities in a Population Exposed to Selenium via Drinking Water, Journal of Toxicology and Environmental Health, 1:729-734 (1980)

16. ABSTRACT

(Project Officer: Daniel Greathouse)

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT: Release to Public	19. SECURITY CLASS (This Report): unclassified	21. NO. OF PAGES: 7
	20. SECURITY CLASS (This): unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

EPA-600/J-80-410
JOURNAL ARTICLE

SELENIUM CONCENTRATIONS AND GLUTATHIONE PEROXIDASE ACTIVITIES IN A POPULATION EXPOSED TO SELENIUM VIA DRINKING WATER

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Selenium concentrations in blood, urine, hair, and tap water were determined in samples obtained from individuals exposed to varying amounts of the element via water from home wells. Glutathione peroxidase activities were also determined on the blood samples. Correlations of blood Se with the enzyme activity were not statistically significant. Correlations of water Se, urine Se, and hair Se with glutathione peroxidase activity were also not statistically significant. It is concluded that a relationship between Se and glutathione peroxidase activity does not exist when Se status is adequate.

INTRODUCTION

Glutathione peroxidase (GSH-px), an enzyme that contains Se, has been extensively investigated over the past 10 yr. One area of research has been the response of this enzyme's activity to dietary intake of Se. Smith et al. (1974) and Omaye and Tappel (1974) found with rat and chick, respectively, that the activity of GSH-px in erythrocytes increases in proportion to the logarithm of the dietary Se concentration. Similar findings by other investigators for bovine and rat erythrocytes have been published (Hafeman et al., 1974; Allen et al., 1975).

To our knowledge, there have been no previous reports on human GSH-px activity in response to dietary intake or to environmental exposure to Se. Only recently have reports been made on the relationship between the enzyme's activity and Se levels in human blood; in these studies, conflicting conclusions were reached. Perona et al. (1977) compared erythrocyte GSH-px activity and plasma Se concentration in 14 patients, 7 of whom had iron deficiency. They found a significant correlation between plasma Se and the enzyme activity. Thomson et al. (1977) also found a significant correlation between whole blood Se and

This investigation was supported by research grant R-803798-01 from the U.S. Environmental Protection Agency. The research protocol was approved by the UCLA Human Research Committee.

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Journal of Toxicology and Environmental Health, 6:731-736, 1980
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whole blood GSH-px activity in a population of New Zealanders; however, in the subpopulation with Se concentrations greater than 10 μg per 100 ml, they suggested that no correlation seemed to exist between the element and the enzyme activity. In contrast, Schmidt and Heller (1976) determined that enzyme activity and Se concentrations are not correlated in erythrocytes. They postulated that only 10% of the total Se content of human erythrocytes is fixed in the enzyme, so that no correlation would be expected.

In these reports comparing Se concentration to GSH-px activity in human blood, the Se concentrations were generally less than 10 μg per 100 ml of whole blood. This paper explores the relationship between whole blood Se and erythrocyte GSH-px activity when whole blood Se levels represent a nondeficient status—that is, levels greater than 10 μg per 100 ml. In addition, the relationship of enzyme activity to urinary excretion of Se, to hair levels, and to increasing gradients of Se exposure via drinking water is examined.

METHODS

A study population consisting of 33 residents from a small community in Milan, New Mexico, was selected for erythrocyte GSH-px activity and Se measurements. These residents consumed drinking water from individual wells; Se concentrations ranged from 26 to 1800 μg/l. Presumably, each well had become contaminated from a uranium mill tailing pond nearby.

Sample collection was conducted over a 4-d period. Scalp hair, blood, a 24-h urine specimen, and a sample of tap water were obtained. Hair and urine samples could not be obtained from each person, which explains the variability in sample sizes.

Participants were questioned about the source of their water, consumption of garden produce, and use of Se-containing shampoo. Only participants were included who had used their well water supply for at least 1 yr. Participants who used shampoo containing Se were excluded from analyses where hair Se concentrations were used. Data obtained for participants using home gardens were analyzed separately for intake correlations.

Sample preparation consisted of a thorough washing of the entire hair samples with nonionic detergent, ethyl alcohol, and a distilled-deionized water rinse. The total amount of each hair sample was used, since in order to be included in the study, each participant had to have lived in the community at least 1 yr. Thus, a steady state with respect to intake and excretion of Se was assumed. Hair samples so prepared and whole blood, urine, and water samples were ashed with nitric and perchloric acid.

Selenium analysis was performed according to a modified method of Kang and Valentine (1977), using 6 *N* HCl instead of a 20% HCl solution

to dilute the digests. From the digests, hydrogen selenide gas was generated and analyzed by atomic absorption spectrophotometry.

To check the sensitivity and accuracy of our analytical method for Se, duplicate samples of a trace metal reference standard prepared by the U.S. Environmental Protection Agency (EPA) were analyzed in each measurement. In all cases, accuracy was within ±5% of the EPA value. The mean recovery percentages of added Se from samples of hair, blood, urine, and water were 84, 88, 94, and 97, respectively.

Erythrocyte GSH-px activity was determined by a modification of the method described by Hafeman et al. (1974), in which the enzyme activity is estimated by measuring the amount of reduced glutathione (GSH) remaining in an incubation mixture at 1 and 3 min. At the collection site, fresh whole blood was washed three times with 3 ml saline phosphate buffer (pH 7.4), then resuspended and hemolyzed with 3 ml distilled-deionized water. This procedure allowed the storage of frozen hemolysate for at least 7 d without a significant decrease in enzyme activity.

For the enzyme assay, 0.1 ml hemolysate prepared as above was mixed with 1 ml 2 m*M* GSH, 0.5 ml 0.01 *M* sodium azide, and 2.4 ml 0.4 *M* sodium phosphate buffer (pH 7.0) containing 4×10^{-4} *M* EDTA. After a 5-min preincubation at 37°C, 1.0 ml 1.25 m*M* H_2O_2 (prewarmed to 37°C) was added to the mixture to initiate the enzyme reaction. At 1 and 3 min after the H_2O_2 addition, 1.0-ml portions of incubation mixture were removed and added to 4.0 ml metaphosphoric acid precipitating solution. GSH in protein-free supernatant was determined by mixing 2 ml supernatant with 3 ml 0.4 *M* sodium phosphate buffer (pH 7.0) and 1.0 ml of 5,5′-dithiobis(2-nitrobenzoic acid) reagent, which contained 0.04% (w/v) 5,5′-dithiobis(2-nitrobenzoic acid) in a 1% (w/v) trisodium citrate solution. The absorbance of the yellow color that developed was measured by spectrophotometry at 412 nm.

A blank (water substituted for hemolysate) was carried simultaneously through the same procedure to measure nonenzymatic GSH oxidation by H_2O_2. The unit of enzyme activity was a decrease in log GSH of 0.001 per minute per milligram of hemoglobin after the decrease in log GSH per minute of the nonenzymatic reaction was subtracted. Thirty replicate measurements of a blood sample were made; the coefficient of variation was 13.4%.

RESULTS

Erythrocytic GSH-px ranged from 2.3 to 18.3 activity units per milligram of hemoglobin, with a mean value of 11.1 ± 4.73 ($n = 33$). The mean and standard deviation for Se concentrations in whole blood for 33 samples was 17.12 ± 2.53 μg per 100 ml, with a range of 13.3 to 24.8 μg per 100 ml. Hair Se concentrations for 30 samples produced a mean and standard deviation of 0.46 ± 0.44 μg/g, with a range of 0.02–1.98 μg/g.

For 28 urine samples the mean and standard deviation were 79.21 ± 48.12 μg/d, with a range of 14.4–337.5 μg/d. Well water samples had Se concentrations ranging from 26 to 1800 μg/l; mean and standard deviation = 327.46 ± 549.1 μg/l.

The relationship between whole blood Se concentration and GSH-px activity in whole blood is illustrated in Fig. 1. A correlation coefficient of −0.193 was obtained, which was found not to be statistically significant ($p > 0.05$). For assessment of exposure, urinary Se concentrations with GSH-px activities and hair Se concentrations with GSH-px activities produced coefficients of −0.195 and −0.189, respectively. Both coefficients were found not to be statistically significant ($p > 0.05$). The correlation coefficient obtained for GSH-px activities with Se concentrations in water also was not statistically significant ($r = -0.024$, $p > 0.05$). Six participants were found to use their home garden as a major source of vegetables. Student's t-test for a comparison of mean GSH-px activities of those who used garden produce to those who did not revealed no statistically significant difference between their respective means of 12.65 ± 3.91 and 10.75 ± 4.89 ($t = 0.88$, $p > 0.05$). The correlation coefficient for the 27 non-garden produce users was also not statistically significant ($r = 0.05$, $p > 0.05$) for an evaluation of the relationship of water Se concentration with GSH-px activity.

DISCUSSION

Glutathione peroxidase has been assessed in various health and disease states of individuals. However, only recently have both the enzyme and Se been determined in the same individuals. These reports, where Se concentrations and enzyme activities were determined, were for cases where

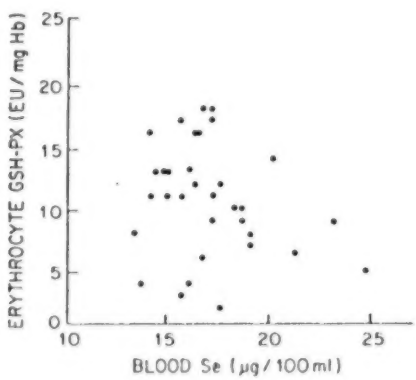

FIGURE 1. Erythrocyte glutathione peroxidase activity as a function of whole blood Se concentrations.

increased exposure had not occurred; indeed, there might have existed a dietary deficiency in Se. In these studies a positive correlation between enzyme activity and Se was determined.

We assessed the relationship between GSH-px activity and Se concentration where an adequate level of Se existed and where increases in exposure to Se had occurred. No increase in GSH-px was noted with increasing blood Se. We showed previously (Valentine et al., 1978) that Se concentrations in hair and urine were correlated with degree of Se exposure. The GSH-px activities, however, were not correlated with the Se concentrations in these body samples or with the varying amounts of Se in the drinking water.

Ganther et al. (1976) state that homeostatic regulation of Se may be limited to a rather narrow range above the Se requirement, and in this range, plateauing of GSH-px in relation to Se intake may occur until the effective homeostatic regulation is exceeded. In our previous study (Valentine et al., 1978), we found that blood Se exhibited a homeostatic mechanism at levels around 20 μg per 100 ml. Thus, when intake levels of Se increased due to exposure to this element in drinking water, no further increase in Se was seen once whole blood Se concentrations reached 20–24 μg per 100 ml.

Two phenomena were observed: (1) increased exposure to Se did not increase GSH-px activity and (2) no correlation was found between Se concentration in blood and GSH-px. Thomson et al. (1977) suggested that no correlation between GSH-px activity and whole blood Se existed when the whole blood Se concentration was greater than 10 μg per 100 ml. They also noted that residents returning to New Zealand have higher Se levels than residents who never left. However, GSH-px levels in both groups were the same. The results of our study of a population with blood Se greater than 13 μg per 100 ml agree with their suggestion that no dramatic increase in GSH-px activity occurs to reflect the greater whole blood Se levels.

CONCLUSIONS

Others have reported that Se levels in blood show a positive relationship to GSH-px activity also measured in blood. We have not been able to demonstrate such a relationship when adequate Se status for a population exists or when large amounts of Se are present in drinking water.

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Received November 6, 1979
Accepted February 20, 1980